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Abbreviations: 8-oxo-7,8-dihydroguanine (8-oxoGua); 8-oxo-7,8-dihydroguanosine (8-oxoGuo); 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo).

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Abstract

Urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) is a widely measured biomarker of oxidative stress. It has been commonly assumed to be a product of DNA repair, and therefore reflective of DNA oxidation. However, the source of urinary 8-oxodGuo is not understood, although potential confounding contributions from cell turnover and diet have been ruled out. Clearly it is critical to understand the precise biological origins of this important biomarker, so that the target molecule that is oxidised can be identified, and the significance of its excretion can be interpreted fully.

In the present study we aimed to assess the contributions of nucleotide excision repair (NER), by both the global genome NER (GG-NER) and transcription-coupled NER (TC-NER) pathways, and sanitisation of the dGTP pool (e.g. via the activity of the MTH1 protein), on the production of 8-oxodGuo, using selected genetically-modified mice. In xeroderma pigmentosum A (*XPA*) mice, in which GG-NER and TC-NER are both defective, the urinary 8-oxodGuo data were unequivocal in ruling out a contribution from NER. In line with the *XPA* data, the production of urinary 8-oxodGuo was not affected in the xeroderma pigmentosum C mice, specifically excluding a role of the GG-NER pathway. The bulk of the literature supports the mechanism that the NER proteins are responsible for removing damage to the transcribed strand of DNA via TC-NER, and on this basis we also examined Cockayne Syndrome mice, which have a functional loss of TC-NER. These mice showed no difference in urinary 8-oxodGuo excretion, compared to wild type, demonstrating that TC-NER does not contribute to urinary 8-oxodGuo levels. These findings call into question whether genomic DNA is the primary source of urinary 8-oxodGuo, which would largely exclude it as a biomarker of DNA oxidation. The urinary 8-oxodGuo levels from the *MTH1*

mice (both knock-out and *hMTH1*-Tg) were not significantly different to the wild-type mice. We suggest that these findings are due to redundancy in the process, and that other enzymes substitute for the lack of MTH1, however the present study cannot determine whether or not the 2'-deoxyribonucleotide pool is the source of urinary 8-oxodGuo. On the basis of the above, urinary 8-oxodGuo is most accurately defined as a non-invasive biomarker of oxidative stress, derived from oxidatively generated damage to 2'-deoxyguanosine.

Keywords: oxidative stress; urine; 8-oxo-7,8-dihydro-2'-deoxyguanosine; nucleotide excision repair; transcription-coupled repair; *MTH1*.

Introduction

The non-invasive assessment of oxidative stress, using nucleic acid-, lipid- and protein-derived biomarkers, has a potential use in studying many major diseases, including cancer, cardiovascular disease, neurodegenerative disease and chronic inflammatory disease [1]. In particular, excreted biomarkers of nucleic acid oxidation may have prognostic use in some conditions, such as radiosensitivity [2], mortality in Type 2 diabetes [3], and survival following radiotherapy [4]. The nucleobase oxidation product 8-oxo-7,8-dihydroguanine (8-oxoGua) is of particular interest and importance, largely because of its biological significance, for example as a replicative and transcriptional mutagenic lesion [5], its modulation of gene expression via affecting transcription factor binding, and DNA methylation; and acceleration of telomere shortening (reviewed in [1]). Furthermore, 8-oxoGua is relatively easy to detect using widely available analytical methodology. Different structural forms of this lesion, consisting of the nucleobase itself (8-oxoGua), the ribonucleoside (8-oxoGuo) and the 2'-deoxyribonucleoside (8-oxodGuo), have been detected in the urine of healthy subjects and patients with various pathologies, but predominantly as 8-oxodGuo, rather than 8-oxoGua. The preference to measure 8-oxodGuo derives from an early study which suggested that urinary 8-oxoGua levels are significantly influenced by diet, whereas 8-oxodGuo levels are not [6], hence the latter became the more favoured urinary biomarker of DNA oxidation, coupled with its relative ease of measurement (via electrochemical detection), and its apparent stability against further oxidation, compared to 8-oxoGua [7, 8]. However, more recent evidence refutes diet as a confounding source of urinary 8-oxoGua in mice [9] and humans [10, 11]. It is widely assumed that 8-oxodGuo is derived from the repair of DNA, and is hence a biomarker of DNA oxidation. In fact, the biological origin of urinary 8-oxodGuo is not known,

limiting our interpretation and understanding of differences and changes in the excretion of this biomarker.

Several repair processes prevent the persistence of 8-oxoGua in the genome, either by acting on DNA directly, or by sanitisation of the dGTP pools (nuclear and mitochondrial). Those acting on DNA include base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), nucleotide incision repair (NIR), and proof-reading by DNA polymerases. To sanitise the dGTP pools, Nudix hydrolases catalyse the hydrolysis of 8-oxo-7,8-dihydro-2'-deoxyguanosine triphosphate (8-oxodGTP), which is a potentially mutagenic DNA polymerase substrate, to 8-oxo-7,8-dihydro-2'-deoxyguanosine monophosphate [7, 12]. The latter is not a substrate for re-phosphorylation, and will not be misincorporated. The activity of BER, predominantly via 8-oxoguanine DNA glycosylase (OGG1), is the primary repair process for removing genomic 8-oxoGua and contributes to urinary 8-oxoGua levels. In contrast, the origins of urinary 8-oxodGuo remain unclear although, as described above, significant progress has been made to exclude contribution from diet [9, 11]. We also cited evidence to argue against cell turnover contributing to urinary lesion levels [11]. Critically, the relative importance of DNA repair pathways in generating free 8-oxodGuo is largely unknown, despite over 25 years of its measurement.

Of all the repair pathways acting on DNA, NER [with the corresponding transcription-coupled NER (TC-NER) and global genome NER (GG-NER) pathways] has the potential to generate 8-oxodGuo, from repair-derived, 8-oxoGua-containing oligonucleotides. Subsequent degradation of these lesion-containing oligonucleotides will release free 8-oxodGuo, in a manner analogous to the processing of cyclobutane thymine dimer-containing oligonucleotide repair

products [13-15]. Indeed, there is considerable evidence to support a role for NER (and some evidence for TC-NER [16-18]), and NER-initiating proteins in the repair of 8-oxodGuo, in various species [19-22]. We have proposed previously that Nudix hydrolases, via degradation of 8-oxodGTP, or 8-oxodGDP, to 8-oxodGMP, and subsequent de-phosphorylation, are an alternate source of extracellular 8-oxodGuo [12]. Indeed, there is some evidence in the literature to support our hypothesis, and to indicate that dGTP in the nucleotide pools is an important target for oxidants. For example, knock-down of *MTH1* (a.k.a. *NUDT1*) decreases 8-oxodGuo excretion in response to ionising radiation [23] and exogenous expression of *MTH1* increases baseline excretion of 8-oxodGuo [24]. Thus the nucleotide pool, and its attendant sanitising activities, would appear to be a credible process for producing urinary 8-oxodGuo [7].

Clearly, determining the exact 'DNA' repair origins of this widely measured urinary nucleic acid oxidation product is critical to enable full interpretation of its measurement, and understand which target molecule is being oxidised. In the present study we have assessed directly the whole body contributions of NER (TC-NER and GG-NER) and MTH1 activity on the genomic DNA levels of 8-oxodGuo, and the production of urinary 8-oxodGuo, in selected genetically-modified mice. The data rule out the contribution of NER, which includes TC-NER and GG-NER, to the levels of urinary 8-oxodGuo.

Materials & Methods

XPA, XPC knock-out and CSB mutant mice.

XPA, *XPC* knock-out and *CSB* mutant mice (C57Bl/6J background) mice were housed in conventional cages with a 12:12-h light-dark cycle and had free access to food and water. All animal experiments were approved by the institutional ethics committee on animal care and experimentation at Leiden University Medical Center. Animals were bred at Leiden University Medical Centre using the methodology described in van Oosten et al. [25]. The *CSB* animals produce a small truncated protein, devoid of biological function, analogous to the common *CSB* defect in humans, resulting in a loss of functional TC-NER; the *XPA* and *XPC* animals lack their corresponding genes. *XPA* mice are deficient in both GGR and TC-NER, and *XPC* mice are deficient in GGR only. Urine samples were collected using a similar protocol to that reported for the *MTH1* mice (below). At the end of the experiment all surviving animals were culled and organs removed and frozen as described for *MTH1* knock-out mice.

MTH1 knock-out mice

Three male *MTH1*$^{+/-}$ mice (backcrossed to a C57BL/6J background for 21 generations; from Division of Neurofunctional Genomics, Kyushu University, Fukuoka, Japan), were mated at the Institute of Cancer Research with female C57BL/6J mice and the offspring genotyped (from tail DNA) as described by Sakumi et al. [26]. Mice thus identified as *MTH1*$^{+/-}$ were mated and their offspring, which included wild-type, *MTH1* $^{+/-}$ and *MTH* $^{-/-}$ mice, were identified by genotyping. At approximately 16 weeks of age the mice were housed individually for 24 h once every four weeks

in metabolism cages, and their urine collected and frozen prior to analysis. The mice sampled were as follows: three male and three female C57BL/6J (wild-type); 3 male and 2 female *MTH1*$^{+/-}$ mice; 3 male and 2 female *MTH1*$^{-/-}$ mice. Twenty-four-hour urine samples were collected a total of 13 times (over 48 weeks) except for two mice that were culled before the end of the experiment (one male wild-type and one female wild-type) due to poor condition. At the end of the experiment all surviving animals were culled and selected organs (brain, heart, kidneys, liver, and lungs) removed and snap frozen in liquid nitrogen. All animal experiments were reviewed and approved by the local ethical committee, and licensed by the UK Home Office.

Human MTH1 transgenic mice

A more limited set of urine samples were obtained from human *MTH1* transgenic (*hMTH1*-Tg) mice (bred and housed at Istituto Superiore di Sanità) comprising 24 h urine samples from at least six animals pooled according to genotype [wild type mice and *hMTH*1-Tg mice [27] and collected at 3, 12 and 24 months of age.

Analysis of urinary 8-oxodGuo, 8-oxoGua and creatinine.

The urinary 8-oxodGuo concentrations were assessed in all the *MTH1* animals at each time point, subject to the availability of sufficient volumes of urine. Urine production over 24 h during months 7-13 in these animals was consistently lower than for months 1-6, resulting in much less urine available for chromatographic analysis, requiring pooling of the urine samples

based on genotype. Urinary 8-oxodGuo and 8-oxoGua were determined in all *MTH1* knock-out mouse urines using GC-MS following HPLC pre- purification [10].

Urinary creatinine concentrations were determined by HPLC-UV detection according to the method of Yang (1998) [28]. Briefly, prior to the chromatographic separation and analysis, urine samples were thawed and diluted 1:10 in distilled water. After centrifugation at 2000 x *g* for 10 min, a 10 µL aliquot was injected onto the HPLC system. The mobile phase consisted of 0.02 mol/L KH_2PO_4 adjusted to pH 6.5 with sodium hydroxide, in 2% (v/v) methanol. The solution was filtered through a 0.22 µm membrane before use. Chromatographic analysis was performed on a Phenomenex Luna C18 column (250 x 4.6 mm, 5 µm) with flow rate 1 mL/min, using a gradient elution reaching 50 % methanol, with a total separation time of 15 min per sample. The eluent was monitored for UV absorbance at 236 nm. Creatinine quantification was based on a four point calibration curve.

Urinary 8-oxoGua was assessed in the GG-NER/TC-NER deficient mice (*CSB*, *XPA* and *XPC*) in the first and last urine collections for each animal, using the above HPLC pre-purification GC-MS technique. Urinary 8-oxodGuo was assessed in the *CSB*, *XPA* and *XPC* animals at all timepoints using a recently reported UPLC-MS/MS method, with sample clean-up by solid-phase extraction [29]. Correction of 8-oxodGuo values for urine concentration was, in this instance, performed by specific gravity (SG) determination using a hand-held refractometer (Reichert Technologies, Depew, NY); since undiluted urine samples gave SG determinations outside the specifications of the instrument, urine samples were diluted 2-, 4- and 8-fold with water and the SG of the undiluted urine determined by extrapolation on the y-axis of a plot of SG versus urine dilution. Correction of urinary 8-oxodGuo concentration was achieved using the following formula [30]:

$8\text{-oxodGuo}_c = 8\text{-oxodGuo}[(pSG-1)/(sSG-1)]$

Where: 8-oxodGuo_c = corrected 8-oxodGuo concentration in pmol/mL; 8-oxodGuo = uncorrected 8-oxodGuo concentration; pSG = population specific gravity (mean SG for a particular genotype over the entire collection period); sSG = individual sample specific gravity.

Genomic 8-oxodGuo analysis

Snap-frozen brain, heart, lungs, liver and kidneys were stored at -80 ˚C until DNA extraction. These were processed for 8-oxodGuo determination in all of the *MTH1* knock-out mice and a selection of the *XPA*, *XPC* and *CSB* animals.

Extraction of DNA from 90-150 mg of homogenised tissue (outer layer removed with a scalpel; homogenised in a hand-held homogeniser) was performed according to a protocol recommended by the European Standard Committee on Oxidative DNA Damage (ESCODD) designed to minimise adventitious formation of 8-oxodGuo [31]. Extracted DNA was dissolved in nuclease-free water overnight at room temperature on a rotating mixer, then quantified by a Nanodrop 1000 device (ThermoFisher Scientific, Loughborough, UK). A volume equivalent to 50 µg DNA was dried in a SpeedVac, reconstituted in DNA digestion buffer, enzymatically digested and analysed by HPLC-MS/MS according to the protocol reported by Singh *et al.* [32]. Heart tissues consistently yielded low amounts of DNA and were not analysed further.

Statistical analysis

Data were plotted and analysed (ANOVA) using GraphPad Prism, v.6.02 (GraphPad Software Inc., La Jolla, CA).

Results

Urinary 8-oxodGuo and 8-oxoGua concentrations in repair-deficient and wild-type mice

The urinary 8-oxodGuo and 8-oxoGua concentrations (9.93 +/- 1.2 and 497.0 +/- 68.0 nmol/mmol creatinine, respectively) in wild type mice were comparable with those reported elsewhere in the scientific literature and, importantly, creatinine, and specific gravity values did not vary significantly within each genotype over the collection period. As reported elsewhere [33], urinary 8-oxoGua values were significantly greater than urinary 8-oxodGuo values.

XPA, XPC knock-out and CSB mice

No statistical differences were seen between the concentrations of 8-oxodGuo, corrected for specific gravity, month-by-month, between any of the genotypes, although there was some evidence of a general upward trend with time (Fig. 1 A-D). For the *CSB* mice, in particular, and also the *XPA* mice, this trend became more pronounced from month seven, becoming statistically significant ($p < 0.05$) at month ten (Fig. 1B and C). Urinary 8-oxoGua concentrations, corrected for creatinine, were also determined in the first and last urine collections (ages one and ten months) for the *CSB*, *XPC* and *XPA* mice, but were not statistically different between each mutant genotype and the corresponding wild-type animals (Supplemental Data Table 1).

$MTH1^{+/+}$, $MTH1^{+/-}$, $MTH1^{-/-}$ and hMTH1-Tg *mice*

No statistical differences were seen in the 8-oxodGuo concentrations between the three genotypes over months one to six, for which urinary 8-oxodGuo values were derived from individual animals. This trend continued for the remainder of the samples, where analysis of samples pooled by genotype was performed (Fig. 2A). Again, there were no statistically significant differences between mice of different genotypes. A similar trend was also seen for 8-oxoGua excretion, with no statistical changes (Fig. 2B). In a sub-study (n=6 mice/group), we also observed no effect on 8-oxodGuo excretion in the *hMTH1*-Tg mice, compared to wild type (Fig. 2C). We did note a ~66% decrease in urinary 8-oxodGuo between 12 and 24 months, in both genotypes.

Genomic 8-oxodGuo levels in repair-deficient mice

Our aim was to examine whether deficiencies in specific repair pathways led to alterations in genomic levels of 8-oxodGuo (expressed as a ratio between the number of 8-oxodGuo per 10^6 dGuo) in a number of key tissues.

XPA, XPC knock-out and CSB mutant mice

No statistically significant differences were noted between genomic levels of 8-oxodGuo in liver, kidney, brain and lung between any of the genotypes, including wild-type, or indeed

between the different tissue types (Fig. 3). The yield of DNA from extractions of heart was too low to allow reliable determination of 8-oxodGuo.

$MTH1^{+/+}$, $MTH1^{+/-}$, $MTH1^{-/-}$ and hMTH1-Tg *mice*

Levels of genomic 8-oxodGuo were higher for kidney tissue compared to brain tissue for all genotypes, in contrast to the findings for *XPA*, *XPC* and *CSB* mice. We noted no relationship between genotype and relative level of lesion. For example, *$MTH1^{-/-}$* mice did not have significantly more 8-oxodGuo in kidney or brain DNA compared to *$MTH1^{+/+}$* mice (data not shown). The low levels of DNA recovery from other tissues precluded reliable and consistent measurement of 8-oxodGuo.

Discussion

Measurement of urinary 8-oxodGuo has received extensive study with a literature database comprising of over 925 articles across a period of 28 years, in which it is commonly referred to as biomarker of oxidatively generated damage to DNA, and released via the action of DNA repair. In fact, the exact origins of this lesion continue to remain obscure, although we have shown that contributions from diet and cell turnover are negligible. This is the first report of DNA and urinary 8-oxodGuo and 8-oxoGua measurements in, and comparisons between, *XPA*, *XPC*, *CSB*, *MTH1*$^{-/-}$, *MTH1*$^{+/-}$, *MTH1* knock-in and *MTH*$^{+/+}$ (wild-type) mice; and their use to examine the relative importance of rationally selected DNA repair pathways in the production of urinary 8-oxodGuo. [7]. In order to ensure our findings are robust, it is important to consider them in the context of the adequacy of the knockout/transgenic models we have chosen, as such genetic manipulation of mice has reported limitations [34, 35]. Reassuringly, knockout of the *CSB*, *XPC* or *XPA* genes confers no consequences for the mice during development unlike, for example, the *ERCC1* knockout mouse, which has a severe aging phenotype. The same is true for the *hMTH1*-Tg mice over the period of time for which we studied the mice. However, it should be noted that, as all the mice are viable, it is plausible that alternative repair pathways are selected for, that are secondary, or back-up processes in wild type mice.

In the present study, deficiencies in NER [*XPA* mice (no TC-NER or GG-NER); *XPC* mice (no GG-NER)] and TC-NER (*CSB* mice) did not alter the excretion of either 8-oxodGuo, or 8-oxoGua, ruling out these processes as sources of urinary 8-oxodGuo and 8-oxoGua. This refutes earlier evidence that 8-oxoGua can be a substrate for NER in humans [20, 22], at least in the context of

fully competent BER, where OGG1 activity is primarily responsible for removing genomic 8-oxoGua [36-39]. Other processes acting on DNA, such as MMR, NIR and exonuclease activities of DNA polymerases, may be a source of urinary 8-oxodGuo, although the likelihood is low and perhaps only relevant to 8-oxoGua in relatively infrequently occurring genomic contexts or under certain cellular conditions. The levels of genomic 8-oxodGuo measured in our study in the various tissues of *XPA*, *XPC* and *CSB* mice are comparable with levels observed in other published studies and notably not significantly different from wild type tissues or cells, where differences only become apparent after oxidant exposure [40, 41]. This further supports our conclusion that not only does NER not contribute to urinary 8-oxodGuo levels, but also 8-oxoGua is not a major substrate for this pathway.

The situation for TC-NER is less well defined, not least because the TC-NER of non-bulky, oxidatively modified nucleobases is somewhat controversial. The blocking of transcription elongation by RNA polymerase (RNAP) is the trigger for TC-NER and our data, coupled with evidence that RNA polymerases may bypass 8-oxoGua [42-44], would appear to strengthen the proposal that 8-oxoGua is not a substrate for TC-NER. However, a recently proposed model suggests inter-play between BER and NER. Specifically, that OGG1 is initially responsible for the removal of 8-oxoGua, and it is the resulting abasic site which, when encountered by RNAP, causes stalling and recruitment of transcription factors [16]. The rest of the repair process is performed by NER. In this model, a defective TC-NER would have no effect on 8-oxoGua removal, as BER is still effective, and the lesion excreted would be the nucleobase 8-oxoGua. Taken together these data further support the importance of OGG1 in removing genomic 8-oxoGua. Indeed studies in OGG1 knock-out mice show a significant (26%) decrease in urinary 8-oxoGua excretion compared

to wild-type animals [33]. However even in these animals excretion is not completely abrogated, perhaps reflecting the adaptation of cells to the loss of a single repair pathway, particularly when this deficit is imposed from conception, and for which numerous back-up pathways have been identified [45]. Our genomic 8-oxodGuo levels also show that defects in the downstream pathways of TC-NER do not affect global genome levels of 8-oxodGuo, adding weight to the above model [16].

The Nudix hydrolase activities of MTH1 (NUDT1) and MTH2 (NUDT15), acting on 8-oxodGTP, and MTH3 (NUDT18) acting on 8-oxodGDP, produce 8-oxodGMP. Subsequent degradation of 8-oxodGMP, by 3'(5')-nucleotidase(s), would generate 8-oxodGuo [7, 46, 47]. Based upon the literature, and our above findings, these activities appear to be candidate sources of urinary 8-oxodGuo (in the absence of any other identified enzyme activities that yield 8-oxodGuo as a product). In the present study, loss of both copies of the *MTH1* gene did not alter the excretion of 8-oxodGuo, compared to wild-type mice, suggesting that MTH1 is unimportant in producing urinary 8-oxodGuo. However MTH2, albeit with decreased activity towards 8-oxodGTP, could act as a 'backup' for MTH1 [46, 48, 49], although its role as a physiologically relevant 8-oxodTPase has recently been questioned [50], particularly in animals that have developed from conception in the absence of MTH1. Furthermore, a contribution from MTH3 activity to urinary 8-oxodGuo production cannot be ruled out. The concept of redundancy in DNA repair pathways is well established (reviewed by Evans et al [45]). The possibility also exists that other, as yet undescribed, 8-oxodGTPase activities may be present in cells that could substitute for MTH1. Indeed, some residual 8-oxodGTPase activity was noted in the crude liver extract from *MTH1*$^{-/-}$ mice [51]. The significantly diminished excretion of 8-oxodGuo into cell

culture supernatant, following ionising radiation challenge, using cells where *MTH1* expression is knocked down [52] or significantly increased excretion of 8-oxodGuo from cells where *MTH1* is overexpressed [53], support our proposal that Nudix hydrolases are a primary source of urinary 8-oxodGuo. Our *hMTH1*-Tg mice did not show any increased 8-oxodGuo excretion because, under baseline conditions, the amount of damage in the pool is the limiting factor for how much 8-oxodGuo is excreted, not the amount of enzyme.

We conclude that neither GG-NER nor TC-NER activities contribute to the production of urinary 8-oxodGuo, on this basis, it may be difficult to confidently define urinary 8-oxodGuo as a biomarker of genomic DNA oxidation. Furthermore, the current findings show that genomic 8-oxoGua is not a prominent substrate for GG-NER or TC-NER. From the present study, it cannot be determined whether or not the 2'-deoxyribonucleotide pool is the source of urinary 8-oxodGuo, although it remains a most plausible source, under physiological conditions. Therefore urinary 8-oxodGuo is most accurately described as a non-invasive biomarker of oxidative stress, derived from oxidatively generated damage to 2'-deoxyguanosine.

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Figure Legends

Figure 1. Urinary excretion of 8-oxodGuo in **(A)** wild-type, **(B)** *CSB* (TC-NER deficient), **(C)** *XPA* (GG-NER, and TC-NER deficient), and **(D)** *XPC* (GG-NER deficient) mice. Bars represent the mean (± SEM) for monthly levels of urinary 8-oxodGuo, corrected for specific gravity. In the case of (B) *CSB*, * indicates significantly different ($p<0.05$) compared to months 1 to 6; and in the case of (C) *XPA*, * indicates significantly different ($p<0.05$) compared to month 1.

Figure 2. (A) Urinary excretion of 8-oxodGuo in $MTH1^{+/+}$ (X), $MTH1^{+/-}$ (□) and $MTH1^{-/-}$ (▲) mice. Mean monthly levels of urinary 8-oxodGuo, corrected for creatinine. Months 1-6, mean (± SEM) for n = 5 or 6 animals; months 7-13 represent urines pooled by genotype prior to analysis, due to small excretion volumes. **(B)** Urinary excretion of 8-oxoGua in $MTH1^{+/+}$ (X), $MTH1^{+/-}$ (□) and $MTH1^{-/-}$ (▲) mice. Mean monthly levels of urinary 8-oxoGua, corrected for creatinine. Months 1-6, mean (± SEM) for n = 5 or 6 animals, however months 7-13 represent urines pooled by genotype prior to analysis, due to small collection volumes. **(C)** Urinary excretion of 8-oxodGuo in *hMTH1*-Tg and wild type mice. Bars represent the mean levels of urinary 8-oxodGuo, corrected for specific gravity, at 12 and 24 months of age.

Figure 3. Genomic levels of 8-oxodGuo in the **(A)** Kidney; **(B)** Liver; **(C)** Brain; and **(D)** Lung tissues of wild-type (WT), *CSB*, *XPC* and *XPA* mice. Bars represent the mean level of genomic 8-oxodGuo/10^6 dGuo (± SEM), for tissue samples from between four and six animals per group, at age 13 months.

Figure 1

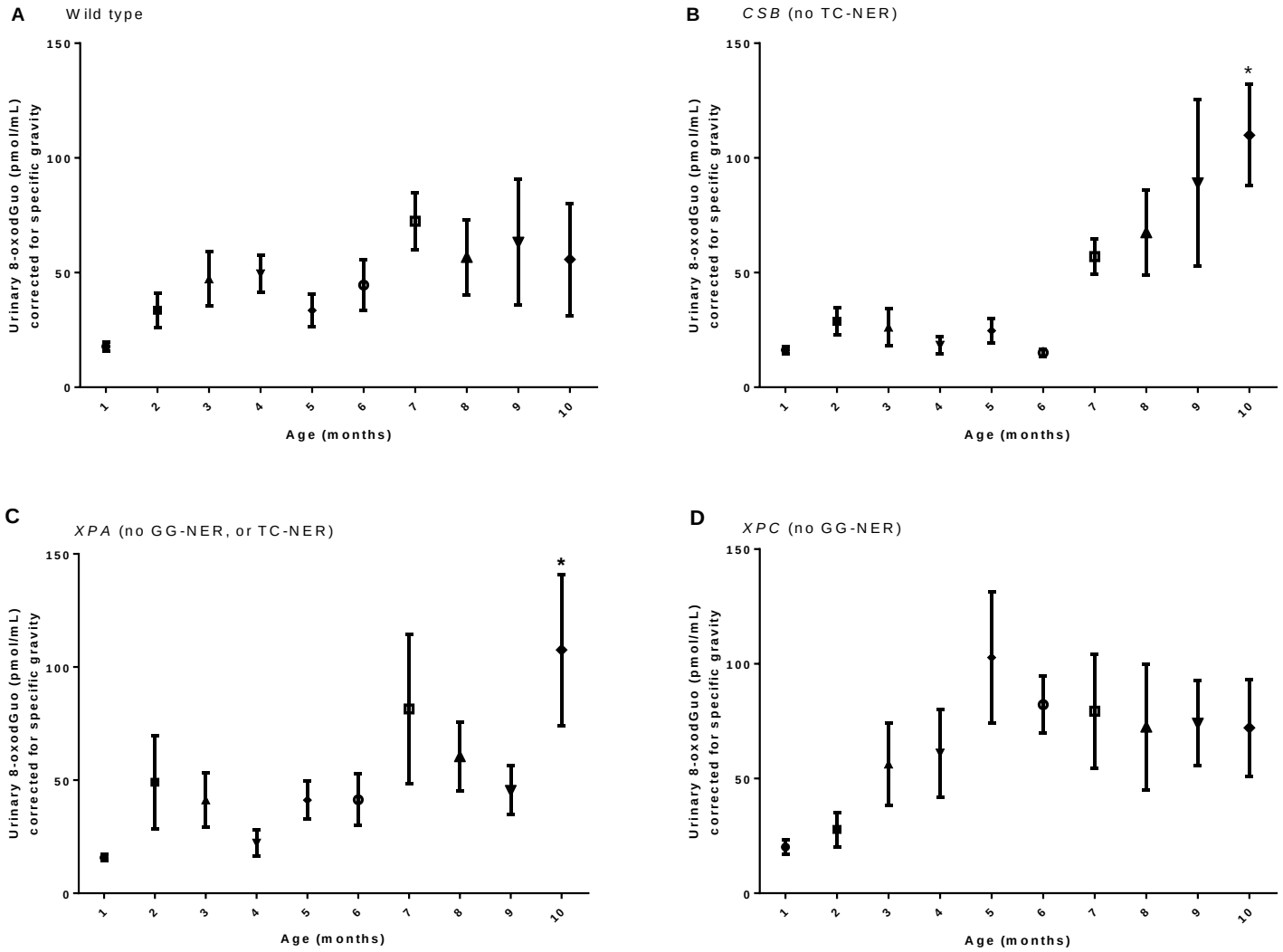

A
Wild type
B
CSB (no TC-NER)
C
XPA (no GG-NER, or TC-NER)
D
XPC (no GG-NER)
Urinary 8-oxodGuo (pmol/mL) corrected for specific gravity
Age (months)
0
50
100
150
1
2
3
4
5
6
7
8
9
10
*

Figure 2

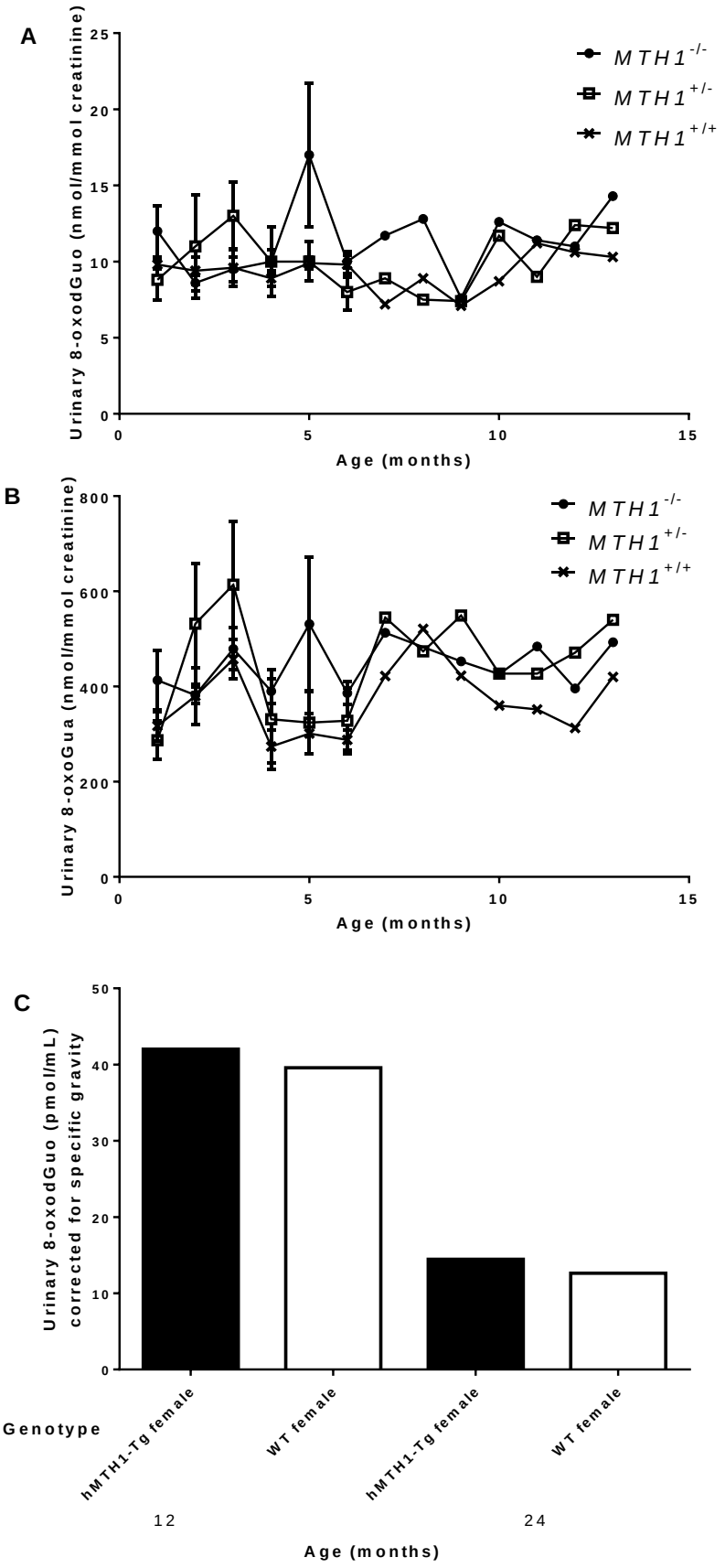
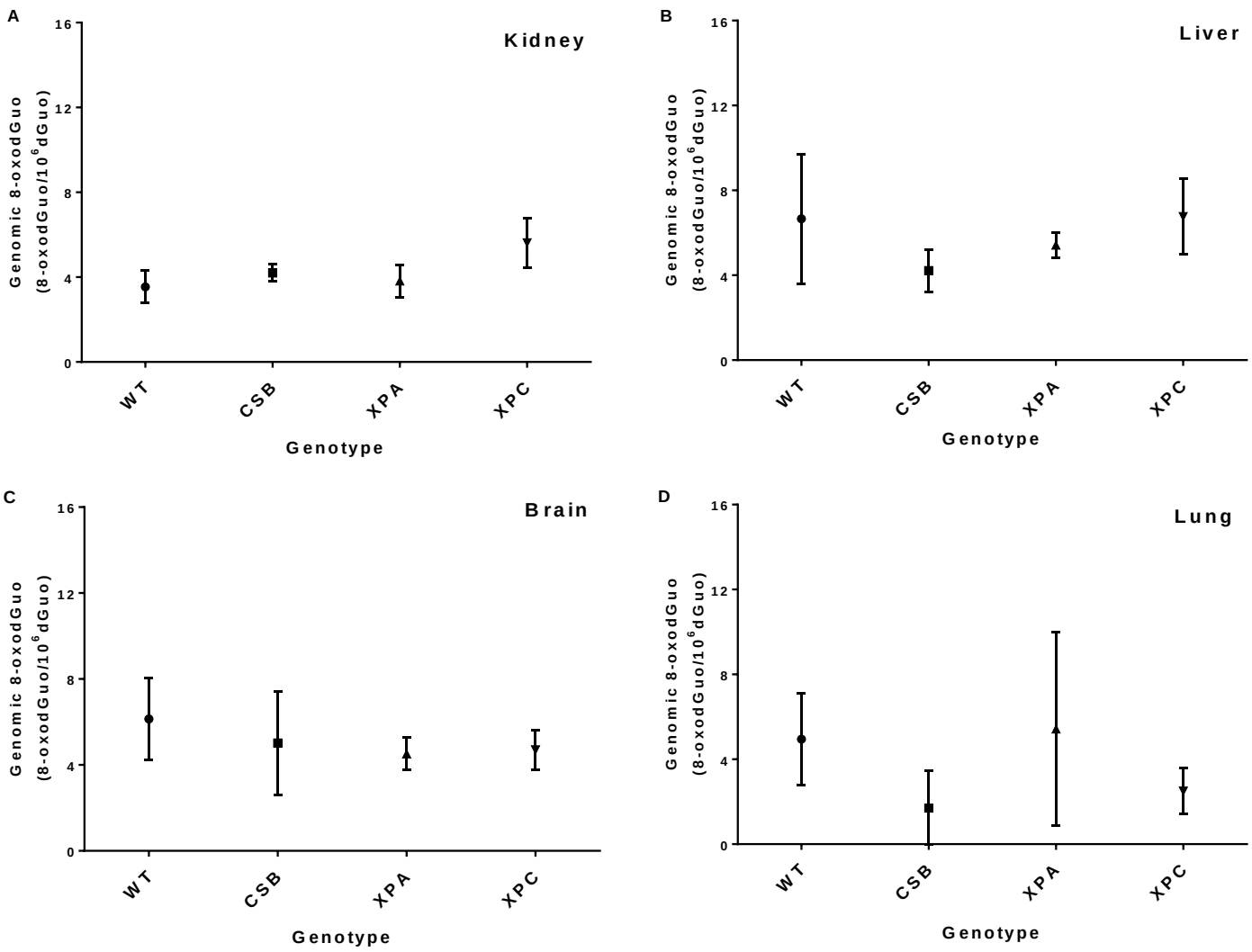

A
Urinary 8-oxodGuo (nmol/mmol creatinine)
25
20
15
10
5
0
0
5
10
15
Age (months)
MTH1-/-
MTH1+/-
MTH1+/+
B
Urinary 8-oxoGua (nmol/mmol creatinine)
800
600
400
200
0
0
5
10
15
Age (months)
MTH1-/-
MTH1+/-
MTH1+/+
C
Urinary 8-oxodGuo (pmol/mL) corrected for specific gravity
50
40
30
20
10
0
Genotype
hMTH1-Tg female
WT female
hMTH1-Tg female
WT female
12
24
Age (months)

Figure 3


A
Kidney
B
Liver
C
Brain
D
Lung
Genomic 8-oxodGuo
(8-oxodGuo/10^6dGuo)
0
4
8
12
16
WT
CSB
XPA
XPC
Genotype

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Supplemental data

SD Table 1. Urinary excretion of 8-oxoGua in wild-type, *CSB* (TC-NER deficient), *XPA* (GG-NER and TC-NER deficient) and *XPC* (GG-NER deficient) mice (n = 4 - 6 mice in each group).

Genotype	**Wild type**		***CSB***		***XPA***		***XPC***	
Sampling timepoint	1 month	10 months	1 month	10 months	1 month	10 month	1 month	10 month
Urinary 8-oxoGua (nmol/mmol creatinine), mean (+/- SD)	497 (68)	344 (86)	413 (92)	324 (84)	415 (97)	288 (71)	692 (332)	(242) 40